Fetal growth and insulin resistance in adult life: role of skeletal muscle morphology


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INTRODUCTION

Men and women who were thin at birth, as indicated by a low ponderal index (PI) (birthweight/length\(^3\)), are insulin resistant [1] and have an increased prevalence of the insulin resistance syndrome – i.e. impaired glucose tolerance, raised blood pressure and dyslipidaemia [2], a finding recently confirmed in a study in Sweden [3]. They also have biochemical abnormalities of skeletal muscle consistent with delayed activation of glycolysis/glycogenolysis at the commencement of exercise [4]. It is thought that babies with a low PI have become undernourished in mid to late gestation but have maintained linear growth at the expense of tissues such as fat and muscle [5]. Because muscle is the main peripheral site of insulin action, these observations have led to the hypothesis that reduced muscle growth in prenatal life may lead to permanent developmental changes in muscle structure and physiology which interfere with insulin action [1].

The nature of these changes is unknown. There is a body of evidence suggesting that morphological abnormalities of skeletal muscle may be involved in the pathogenesis of insulin resistance. Insulin resistance is associated with a shift in muscle fibre composition towards a higher proportion of the relatively insulin-resistant fast-twitch (type 2) fibres at the expense of the insulin-sensitive (type 1) fibres [6–8]. Animal experiments suggest that undernutrition either in utero or during neonatal life may permanently alter muscle fibre composition [9, 10]. In parallel with the fibre type alterations, insulin-resistant or glucose-intolerant subjects may have reduced capillary density [6–8] and abnormalities of capillary structure such as a thickened capillary basement membrane, and these could reduce the rate of diffusion of insulin to its receptor, thereby causing insulin resistance [11]. Alternatively, the insulin resistance and the apparent decrease in glycogenolysis/glycolysis in people who were thin at birth could be due to changes in the activity or regulation of enzymes controlling glucose uptake and metabolism [12].

1. Thinness at birth is associated with insulin resistance in adult life and an apparent delay in activation of glycolysis/glycogenolysis in exercising skeletal muscle. As developmental abnormalities of skeletal muscle histology or metabolism may explain this association we examined muscle histology, biochemistry and blood flow in a group of 27 adult women whose birth details were known.

2. Subjects were examined by near-infrared spectroscopy to determine forearm muscle oxygen supply, and by muscle biopsy and forearm plethysmography. Those with a ponderal index at birth <23 kg/m\(^3\) were insulin resistant (assessed by the short insulin-tolerance test – mean rate constants for glucose disappearance = 4.14 compared with 4.83%/min, \(P = 0.045\)) and had significantly more rapid muscle reoxygenation than the remainder of the subjects (13 compared with 22 s, \(P = 0.004\)).

3. Thinness at birth did not influence muscle capillary density, muscle glycogen content, glycogen synthase activity, citrate synthase activity or resting forearm blood flow.

4. Insulin resistance seen after fetal malnutrition was not associated with abnormal muscle histology, resting muscle blood flow, mitochondrial volume or glycogen content.

5. The increase in muscle reoxygenation rate in adult subjects who were thin at birth could occur to promote oxidative ATP synthesis in compensation for the delay in activation of glycolysis/glycogenolysis. It suggests altered regulation rather than structure of the muscle microcirculation. These changes appear to antedate the structural and biochemical changes seen in muscle from patients with established diabetes.

Key words: glycogen, glycogen synthase, near-infrared spectroscopy, plethysmography.

Abbreviations: MRS, magnetic resonance spectroscopy; NIRS, near-infrared spectroscopy; PI, ponderal index.

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We have therefore investigated the relation between fetal growth and skeletal muscle morphology, blood supply and the activities of key enzymes of glucose metabolism in adult life. These studies were designed to determine whether structural or functional differences in muscle could explain the association between reduced fetal growth and insulin resistance.

SUBJECTS AND METHODS

Subjects

The subjects form part of a larger group of men and women who were born at Sharoe Green Hospital, Preston, Lancashire, U.K., and who have formed the basis for a prospective investigation between fetal and infant growth and diabetes in adult life. This study was carried out in accordance with the Declaration of Helsinki (1989), informed consent was obtained from each subject and the study was approved by the Preston District and Hospital Ethical Committee. Three-hundred and ninety-three adults who were born at Sharoe Green Hospital and who still live in or close to the city were invited to undergo a glucose tolerance test [13], of whom 266 agreed. From the 232 normoglycaemic subjects we selected a sample of 42 men and 40 women to undergo insulin resistance measurements using a short insulin-tolerance test [1]. The insulin tolerance tests were carried out between 08.30 hours and 11.00 hours. Subjects had fasted for 12 h overnight and had been asked to abstain from alcohol and not to smoke during the fasting period. A bolus of human Actrapid insulin (Novo Laboratories, Basingstoke, U.K.), 0.05 units/kg, was injected into an antecubital vein and venous blood was taken from a cannula placed in the dorsum of the hand of the same arm. In order to arterialize the venous blood, the hand was placed in a water bath held at a constant temperature of 43°C for 20 min before insulin injection and kept there until the end of the study. Venous blood was sampled at 0, 3, 5, 7, 9, 11, 13 and 15 min after the insulin injection. The blood samples were kept on ice and analysed for whole-blood glucose with a Yellow Springs analyser (YSI; Yellow Springs, OH, U.S.A.). Linear regression was used to estimate the slope of the decline in log-transformed blood glucose concentration. The slope was multiplied by −100 to derive the rate constant (KITT) which is equivalent to the percentage decline in blood glucose per min [14]. In addition the subjects' heights and weights were measured with a portable stadiometer and digital scales, and their waist and hip circumferences with a steel tape measure. Blood pressure was measured by an automated recorder (Dinamap model 1846X; Critikon, Tampa, FL, U.S.A.) in seated subjects with a cuff of appropriate size placed on the left arm as described previously. Two readings were taken and the average used in the analysis.

Near-infrared spectroscopy (NIRS) measurements

Because the relationships between fetal growth and insulin resistance were stronger in women than men [1], we asked the 40 women to undergo NIRS measurements. In 27 female subjects who agreed, the half-times for tissue reoxygenation after exercise were determined using a commercial apparatus (RunMan; NIM Inc, Philadelphia, PA, U.S.A.) to perform NIRS of flexor digitorum superficialis muscle. The light source was placed over the muscle and reflected light was measured at 760 and 850 nm to distinguish oxygenated and deoxygenated Hb. The NIRS signal arises predominantly from blood vessels less than 1 mm in diameter [15]. The signal is not contaminated by changes in skin blood flow [15]. Finger flexion exercise was performed by lifting a weight of 1.5 kg a distance of 5 cm at a rate of 40/min. After 1 min of exercise a cuff was inflated around the upper arm to 50 mmHg above systolic blood pressure as exercise was continued for a further 20–30 s. There was a 5 s delay between cessation of exercise and deflation of the cuff. The half-time of reoxygenation was determined using the point at which the cuff was deflated as the point of maximal deoxygenation and the start of metabolic recovery (Fig. 1).

Histological measurements

Twenty-six of these 27 subjects agreed to undergo a gastrocnemius muscle biopsy. After an overnight fast the women consumed a standard meal containing 30 g of carbohydrate. One hour later a 1 2 3 4

Fig. 1. Representative tracings from NIRS of the finger flexors of two subjects. Relative oxygenation is expressed for the duration of aerobic and then ischaemic exercise and recovery from ischaemia. Deflection of the trace towards the top of the page signifies relative deoxygenation of the muscle. 1, commencement of finger flexion exercise; 2, commencement of ischaemia, exercise continuing; 3, cessation of exercise, ischaemia continuing; 4, reperfusion. The half-time of reoxygenation of the muscle was taken as a measure of oxygen supply to the tissue. The horizontal line at the bottom right-hand corner represents 1 min.
biopsy, 100–200 mg, was obtained from the medial head of gastrocnemius muscle under local anaesthesia (1% lignocaine) using the conchotome technique [16]. The biopsy was trimmed of fat and connective tissue before being frozen in liquid nitrogen. Cross-sections of muscle (8 μm) were cut with a cryotome at −20°C. Capillaries were stained using a Ulex europaeus lectin stain and the total number in a 2 mm square was counted. Sections were stained for myosin ATPase activity after preincubation at pH 4.3, 4.5 and 9.4. The fibres were classified into slow-twitch (type 1) and fast-twitch (type 2) fibres. In each biopsy, 500 fibres were counted. Fibre surface areas were measured by image analysis on a Leica dialux microscope using Colourvision and PrismView software (Improvision, Coventry, U.K.) on an Apple-Macintosh Quadron 700 computer.

Electron microscopy and measurement of capillary basement membrane width

Muscle biopsies were fixed in 3% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) and stored in fixative. Before processing they were washed overnight in buffer (0.1 mol/l cacodylate plus 0.23 mol/l sucrose), post fixed for 2 h in 2% osmium tetroxide, en bloc stained in 2% aqueous uranyl acetate for 30 min and dehydrated through a graded ethanol series. Biopsies were soaked in Histosol (1:1 trichloroethane and perchloroethylene) for 30 min before infiltrating with Spurr's resin, embedding in Taab capsules (Taab Laboratories, Aldermaston, Berks., U.K.) and polymerization. Sections (0.5 μm) were cut on diamond knives for transmission electron microscopy, picked up on copper grids, counterstained with lead citrate and examined using a Philips 201 or Hitachi H7000 transmission electron microscope. The thickness of the basement membrane was measured at 10 equivalent points along the circumference of 12 consecutive capillaries at a magnification of ×10000 as described previously [11].

Biochemical assay

The following maximal enzyme activities were determined in the muscle biopsies: citrate synthase (EC 4.1.3.7) [17], phosphofructokinase (EC 2.7.1.11) [18] and glycogen synthase (EC 2.4.1.11) activities were determined at 0.1 mmol/l glucose-6-phosphate (initial) and 10 mmol/l glucose-6-phosphate (total) and the result expressed as a percentage (initial/total) [19, 20]. The total glycogen content of the muscle was also determined from the amount of glucose formed after enzymic hydrolysis with amyloligosidase (EC 3.2.1.3) of sonicated cell extracts [21]. Protein content of the muscle was determined by potassium hydroxide digestion of the tissue as described by Miller [22].

Plethysmography

Forearm blood flow was measured by venous occlusion plethysmography with a strain-gauge apparatus applied at a fixed distance from the styloid process. The circulation to the hand was excluded by inflating a paediatric cuff to suprasystolic pressure around the wrist. Blood flow was measured by inflating another cuff about the upper arm to 40 mmHg for 10 s and deflating it for 5 s. The blood flow was measured at least four times.

Statistical measurements

Data are expressed as means (SEM). Significant differences were assumed if P < 0.05 after a Mann–Whitney U-test or Spearman correlation where appropriate.

RESULTS

Since PI was the birth measurement most strongly predicting insulin resistance, the women were divided into two groups: those with a PI at birth below (n = 16) and above (n = 11) the overall mean of 23 kg/m³. The groups did not differ significantly in terms of age (55.3 versus 54.1 years), body mass index (23.5 versus 25.2 kg/m³), body fat percentage (37.2 versus 39.2%) or systolic blood pressure (138.4 versus 144.7 mmHg). The low-PI group was significantly more insulin resistant as assessed by the short insulin-tolerance test (Table 1).

Table 1. Aspects of insulin sensitivity, muscle histology and physiology grouped according to PI at birth. Values are means (SEM). Statistical significance: *P = 0.004, Mann–Whitney U-test compared with low-PI group; **P = 0.045, Mann–Whitney U-test compared with low-PI group.

<table>
<thead>
<tr>
<th>PI at birth</th>
<th>&lt;23 kg/m³</th>
<th>&gt;23 kg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Fasting glucose (nmol/l)</td>
<td>5.3 (0.1)</td>
<td>5.2 (0.2)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>42.1 (3.1)</td>
<td>38.4 (3.3)</td>
</tr>
<tr>
<td>Rate constant of glucose disappearance (%/min)</td>
<td>4.14 (0.29)</td>
<td>4.83 (0.18)**</td>
</tr>
<tr>
<td>Muscle histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Fibres/mm²</td>
<td>121 (2)</td>
<td>115 (2)</td>
</tr>
<tr>
<td>% type I fibres</td>
<td>67 (3.5)</td>
<td>68 (2.5)</td>
</tr>
<tr>
<td>Muscle fibre area (μm²)</td>
<td>9080 (539)</td>
<td>9180 (692)</td>
</tr>
<tr>
<td>Capillary density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillaries/mm²</td>
<td>264 (2)</td>
<td>244 (4)</td>
</tr>
<tr>
<td>Capillaries/fibre</td>
<td>2.3 (0.1)</td>
<td>2.6 (0.0)</td>
</tr>
<tr>
<td>Basement membrane thickness (μm)</td>
<td>233 (12)</td>
<td>250 (18)</td>
</tr>
<tr>
<td>Resting forearm blood flow (ml/min)</td>
<td>5.0 (0.2)</td>
<td>6.8 (0.3)</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Forearm muscle reoxygenation half-time (s)</td>
<td>13 (0.5)</td>
<td>22 (1)*</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 1 also shows the measurements of muscle histology, forearm blood flow and muscle reoxygenation rate in the two groups. The two groups did not differ in fibre density or composition and the capillary density, basement membrane thickness and forearm blood flow were similar in the high- and low-PI groups. NIRS from 2 of the 27 subjects was unsatisfactory due to a poor signal. In the remaining 25 subjects, the mean half-time for reoxygenation was significantly lower in the low-PI subjects ($n = 16$), implying increased oxygen supply to the muscle (Table 1 and Fig. 2). The increased reoxygenation was more closely related to the PI at birth than to the other birth measurements. Thus, for example, reoxygenation rate did not correlate significantly with birthweight ($P = 0.12$), placental weight ($P = 0.23$), head circumference ($P = 0.2$) or gestational age ($P = 0.95$). In regression analyses with muscle reoxygenation rate as the dependent variable and PI at birth, age, body mass index, waist-to-hip ratio and blood pressure as the independent variables, only the association between PI at birth and reoxygenation remained statistically significant ($P < 0.01$). Muscle reoxygenation rate did not correlate with the measured enzyme activities and the correlation of reoxygenation rate with high-energy phosphate metabolism within the muscle during exercise is discussed below. Capillary density, forearm blood flow and reoxygenation did not predict insulin resistance or glucose tolerance in these subjects.

Table 2 shows the relationship between PI at birth and the glycogen content of muscle and the activity of key regulatory enzymes controlling glucose storage or oxidation. There was no difference in citrate synthase, phosphofructokinase or glycogen synthase activity between the two groups (Table 2) and there was no correlation of these activities with measures of the subjects’ glucose tolerance or their birth details. The amount of glycogen in the muscle did not differ between the two groups and was not related to the degree of insulin resistance or the PI at birth.

**DISCUSSION**

Thinness at birth predicts insulin resistance in adult life and this has led to the hypothesis that insulin resistance could be a ‘programmed’ response to early growth restriction [1]. The mechanism of development of this insulin resistance is not clear but the importance of skeletal muscle as a peripheral site of insulin action has implicated skeletal muscle metabolic and morphological abnormalities in this condition, especially as muscle histology is known to be abnormal in subjects with established glucose intolerance or diabetes.

The present data, however, demonstrate the lack of a relationship between body size at birth and muscle morphology, capillary density or forearm blood flow in the adult. They also show the absence of a relationship between insulin or glucose tolerance and capillary density or reoxygenation rate. The absence of a link between fetal nutrition and muscle morphology and glucose intolerance suggests the effect of early growth restriction on carbohydrate tolerance may not be mediated by abnormalities of forearm blood flow or capillary density. It also suggests that insulin resistance *per se* is not characterized by changes in muscle capillary density, muscle glycogen levels, limb blood flow or mitochondrial and glycolytic enzyme maximal activity. Capillary density in obese subjects may be related more to body weight and body fat distribution than to insulin resistance [6], and this may explain why our data conflict with a study that showed insulin-resistant obese subjects have a low capillary density that is inversely related to fasting insulin levels [7].

NIRS was not performed on the gastrocnemius muscle due to poor tolerance of an inflated thigh cuff. We were able to study flexor digitorum super-
biopsy the forearm flexor muscles and hence skeletal ficialis by NIRS in all subjects. We were unable to obtained from the upper limb. The forearm flexor muscles have approximately the same fibre type distribution (50% type 1) as the gastrocnemius muscle (50–70% type 1) [8, 23] and both muscle groups reveal similar metabolic abnormalities in systemic conditions associated with myopathy such as chronic renal or heart failure or mitochondrial myopathy [24–27].

The 27 women in our study are a sub-sample of a larger group of men and women who were born and still live in Preston, and are therefore unrepresentative of all men and women in the town. It is unlikely, however, that this selection would introduce bias as the comparisons in the study are internal. Our measure of insulin resistance was based on the fall in blood glucose after intravenous insulin injection. This method is an imperfect measurement of insulin sensitivity as it measures insulin action and cannot determine insulin resistance; nevertheless, the short insulin-tolerance test correlated highly \((r = 0.81–0.86)\) with estimates of insulin sensitivity using a euglycaemic clamp, widely regarded as the reference method [14, 28, 29]. The fasting insulin concentration, although widely used in epidemiological studies, is a weak measure of insulin resistance.

Our study did demonstrate a significant relationship between muscle oxygen supply and thinness at birth. Subjects with a low PI at birth had faster reoxygenation rates (Fig. 2). NIRS measures the change in tissue oxygenation, i.e. the balance between oxygen supply and consumption. Our data imply either oxygen consumption is decreased in these patients and/or oxygen supply is increased. Oxygen consumption rates (indirectly assessed by citrate synthase activity (Table 2) and the \(^{31}\text{P MRS-derived oxidative ATP synthesis rate} [4]\) appear unaffected by birthweight so a decrease in reoxygenation half-time in these subjects is either evidence that muscle oxygen supply, i.e. blood flow, in these subjects is increased or that there is increased efficiency in oxygen extraction by the muscle. Plethysmography, which measures the combination of skin and muscle blood flow, did not detect an increase in resting forearm blood flow in the low-PI subjects nor was there any correlation of resting forearm blood flow with reoxygenation rate. There may be a change in regulation of blood flow in low-PI subjects during exercise. The maximal blood flow to the muscle, or the blood flow to the muscle after ischaemic exercise, was not measured. The forearm reoxygenation rate was not associated with gastrocnemius muscle capillary density (Table 1). This implies that any change in the regulation of microvascular blood flow and muscle oxygen supply in the low-PI subjects was not associated with a structural change in the vasculature. This altered regulation may have its origin as a tissue response to an underlying metabolic abnormality.

\(^{31}\text{P Magnetic resonance spectroscopy (MRS) has demonstrated an apparent delay in the activation of anaerobic glycolytic metabolism in the flexor digitorum superficialis muscle of low-PI subjects. Specifically, levels of phosphocreatine, a readily available store of high-energy phosphate for phosphorylation of ADP independent of glycolysis, were lower and [ADP], the driving force for mitochondrial (oxidative) phosphorylation, was higher in the low-PI subjects during an exercise protocol which stressed anaerobic/glycolytic metabolism [4]. The [ADP] in the flexor digitorum superficialis muscle during early exercise correlated inversely with PI, suggesting that the drive to oxidative ATP synthesis in early exercise was related to fetal malnutrition. The flexor digitorum superficialis muscle reoxygenation rate correlated significantly with exercise duration and initial changes in phosphocreatine and [ADP] [4]. In the present study, the increase in the flexor digitorum superficialis muscle oxygen supply in low PI subjects is associated with normal resting forearm blood flow and normal mitochondrial function as assessed by \(^{31}\text{P MRS} [4]\). This increased oxygen supply to the forearm muscle detected by NIRS could be evidence of a microvascular adaptation by the muscle, promoting an increase in oxidative ATP production during early exercise and thus helping to restore any deficit brought about by the delay in activation of anaerobic glycolytic/glycogenolytic ATP synthesis. Increased blood flow to muscle during exercise could be an early abnormality in insulin resistance. This finding is consistent with previous work which showed that glucose-tolerant subjects who subsequently develop non-insulin-dependent diabetes mellitus have an increased capillary density in skeletal muscle [30]. Changes in muscle ultrastructure that characterize established diabetes (such as a change in fibre type or decreased capillary density) occur later in the development of glucose intolerance.

As with the muscle histology, muscle biochemistry assayed \textit{in vitro} is altered once glucose intolerance has developed. Oxygen consumption and the maximal activities of oxidative enzymes (i.e. succinate dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and citrate synthase) are decreased in the muscle of subjects with a pathological oral glucose-tolerance test and no obvious vascular disease [12, 30] and in the muscle of streptozotocin-treated rats [31]. This indicates a reduction in the oxidative capacity of skeletal muscle once insulin deficiency is present. Citrate synthase activity was normal in the low-PI subjects. The present study also shows that enzymes important in glucose disposal by the muscle cell, glycogen synthase and phosphofructokinase, have normal maximal activities \textit{in vitro} in non-obese, glucose-tolerant, insulin-resistant subjects with a history of low PI at birth. Glycogen levels are
normal in these subjects, suggesting that a reduced level of intracellular glycogen is not responsible for the apparent delay in activation of glycolysis/glycogenolysis in exercise. In summary, there was no evidence of metabolic abnormality in vitro in this group of insulin-resistant subjects although it is still possible that the regulation in vivo of these enzymes (and others) may be affected by intrauterine events.

In conclusion, insulin resistance associated with a low PI at birth is characterized by a change in regulation of muscle microcirculation rather than a histological change in the muscle. Capillary density, muscle glycogen levels, resting limb blood flow or mitochondrial and glycolytic enzyme activity are not related to intrauterine malnutrition or to insulin resistance per se. Fetal malnutrition is associated with an increase in muscle oxygen supply, possibly an adaptation to a delay in activation of glycolysis/glycogenolysis, and these metabolic and physiological alterations exist before the development of diabetes.

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